

"Increased Stability of the Oxy-
Haemoglobin Molecule as a factor
in the pathogenesis of Diabetes
Mellitus."

by

GEORGE SCOTT CARMICHAEL

M.B., Ch.B., (First Class Honours) 1900.



Glycosuria may well be looked on as a symptom arising from several or many morbid conditions quite distinct in themselves and not bearing of necessity any definite relation to each other :- the disease Diabetes Mellitus implying the presence of one or other of these morbid conditions.

One finds an analogous condition of affairs, for example, in the group of diseases expressing themselves by anaemia - the Etiology and progress of diseases with that symptom showing very great diversity.

Diabetes Mellitus therefore is to be looked on in the present thesis as a disease where Glycosuria is the prominent symptom. This symptom may not and in all probability does not, stand in any etiological relationship with any hitherto recognised cause of its occurrence.

In addition to the glycosuria of Diabetes Mellitus/

Mellitus we have other definite symptoms such as emaciation, reduction of alkalinity of the blood, and elimination in the urine of morbid products of metabolism other than glucose, e.g. Acetone, Diacetic Acid, B oxybutyric acid.

Physiology and General Pathology of Sugar Secretion.

In introducing this subject for consideration it may be well in the first place to examine the Physiology and General Pathology of Sugar Secretion, and the ultimate fate of the carbohydrates ingested in the shape of food.

Beginning first with Starch, one finds that it, like dextrin and glycogen, is a polysaccharide and cannot be absorbed unchanged. The necessary change is brought about by the action of the disease found in the buccal and pancreatic secretions principally.

Changes occur through several stages until maltose is reached. As this latter substance passes through the/

{ distase }

the intestinal villi and portal vein a further change into glucose is undergone.

As regards cane sugar, acids, bacteria and ferments all lend their assistance in breaking up this disaccharid into its two components dextrose and levulose and in that way it is absorbed.

Fruit sugar and milk sugar are said to pass into the portal blood stream unchanged. From the intestine the carbohydrate stream flows along the portal vein to the liver - the blood in this vein containing according to von Mering from .1% in flesh eating animals to .4% - in carbohydrate feeding animals.

In the liver the carbohydrate brought to it is again transformed into the polysaccharid-glycogen and as such or perhaps in loose combination with albumin is stored in the hepatic cells.

In the general blood stream normally, no carbohydrate occurs other than glucose, with the doubtful exception/

exception perhaps of very slight traces of glycogen.

Glycogen occurs elsewhere than in the liver cells. The total bulk of the muscles is said to contain an amount equal to the liver content of glycogen.

From the liver glucose passes in constant quantity into the blood stream whence the muscles get their store.

Now glycogen is produced otherwise than from carbohydrates. In animals rendered glycogen free by starvation injection of albuminates has been found to be followed by the development of glycogen

The conclusions from that experiment, a conclusion amply supported by observations upon the subjects of Diabetes Mellitus, is that the non-nitrogenous part of the large proteid molecule is first broken up into carbohydrate before yielding final products of combustion such as carbon dioxide and/

and water (Minkowski⁽¹⁾)

In health there is no elimination of carbo -
hydrate as such, the cells of the organism consume it
and if the supply of carbohydrate for the necessary
heat and energy production be deficient, the store
of glycogen in the liver and muscle becomes exhausted
and the quantity of glucose in the blood is maintained
at its constant by utilising the albumin of the
muscle with consequent wasting.

Fat is at the same time utilised in maintaining
the supply of material for the metabolic changes
associated with the development of these vital forces
- heat and energy.

Should the carbohydrate be in excess of the
demands upon it, we find results depending upon the
period of time over which that excess has been
ingested.

If a slight but constant excess be given over a
period/

period of some time, we have first an exhaustion of the storage capacity of the liver and muscle for glycogen and coincidently we find a conversion of carbohydrate into fat. This conversion of carbohydrate into fat is of significance in the association of lipaemia with Diabetes Mellitus where such a development is characterised by a simultaneous disappearance or marked diminution in glucose content of the blood . Should the rate of carbohydrate ingestion be great, then we find the condition known as alimentary glycosuria occurring, where the glucose content of the blood has risen beyond 0.2% with consequent elimination of that carbohydrate via the kidneys.

This alimentary glycosuria is a physiological process.

The several recognised morbid conditions associated with glycosuria are of interest and of significance.

We/

We have first, that form of glycosuria typified in the so called "puncture diabetes" of Claude Bernard - a form of diabetes however that occurs in various forms of brain injury. This glycosuria is characterised by a complete discharge of glycogen from the liver and if the liver be glycogen free there is no more glycosuria.

As regards the glycosuria following phloridzin poisoning we find an altogether different condition obtaining. The action of this substance appears, from much and recent observation, to be confined entirely to the kidneys, the blood being consequently poor in glucose.

In the condition following extirpation of the pancreas we find a most significant grouping of symptoms, where along with extreme glycosuria we find the acidosis, emaciation, acetonaemia, symptoms culminating in coma and death - in all aspects therefore/

fore closely resembling in its progress a severe and fatal case of Diabetes Mellitus.

In pancreatic diabetes as in Diabetes Mellitus we have an increased production of glucose from proteid and likewise a diminished consumption of glucose.

Chemical Pathology and Morbid

Metabolism in Diabetes Mellitus.

Glycosuria stands out prominently among the symptoms of Diabetes Mellitus.

A healthy individual can deal with his ingested carbohydrates by burning up part, storing up part and changing part into fat. A diabetic individual excretes carbohydrates. The degree of this non-consumption varies from the elimination of part of the ingested carbohydrates to the elimination of all with in addition, that carbohydrate derived from the partial metabolism of the proteid material.

This/

This fact that in Diabetes Mellitus some sorts of sugar molecules are disposed of better than others is of great significance because it is incomprehensible on the theory of an overproduction of glucose, (of pancreatic and "puncture" diabetes,) but is explicable on the theory of a diminished consumption of that material.

Pathogenesis of Diabetes Mellitus.

In the study of the pathogenesis of Diabetes Mellitus we find that it has been attributed to lesions of many organs, chief among those being the brain, following upon Bernard's discovery; and undoubtedly glycosuria has become established coincidently with the occurrence of various brain and nerve lesions.

Again, following Lancereux, von Mering and Minkowski the pancreas has been given as the seat of the morbid changes associated with the Diabetes Mellitus, - likewise diseases of the liver.

The/

The gouty and rheumatic diatheses also are frequently associated with true Diabetes Mellitus, the relationship however lies hidden in obscurity.

Absence of normal ferment, reduction of the alkalinity of the blood, renal changes, following von Mering's observations upon Phloridzin, have all likewise been given the credit of being the essential pathogenetic factors in the establishment of this disease.

It was formerly believed that the process of oxidation was lowered in cases of Diabetes Mellitus and that the gaseous exchanges in such cases give evidence of such a diminished oxidation - this theory of diminished oxidation receiving support from the accumulation in the blood of partially oxidised substances such as β oxybutyric acid.

The determination of the respiratory change shows the relationship between the output of carbon dioxide/

dioxide and the intake of oxygen, the ratio between the volume of carbon dioxide discharged and oxygen taken in being known as the respiratory quotient. Such a quotient of course indicates how much of the oxygen combines with carbon to form carbon dioxide, one volume of oxygen combining with carbon to yield one volume of carbon dioxide.

It therefore follows that the less oxygen unites with carbon to form carbon dioxide, the ultimate product of complete carbohydrate combustion, the smaller will be the value attached to the respiratory quotient.

An individual living on a mixed diet gives out less carbon dioxide than he receives oxygen, the relationship being on an average 9:10; 0.9 being therefore the respiratory quotient. Various conditions influence the relative proportion of these gases.

This/

This quotient becomes greater when increased amounts of carbohydrates are consumed in the body and it becomes smaller when a larger amount proportionately of albuminoids and fats is consumed.

Weintraud and Laves⁽²⁾ found that in diabetics who were fasting the respiratory quotient was definitely lowered and that contrary to what would be expected in the healthy organism this quotient was not increased by the ingestion of carbohydrates.

Experiments of Laves⁽³⁾ upon healthy men (7 experiments) gave him an average respiratory quotient of 0.872, and experiments by the same author in collaboration with Weintraud upon Diabetic patients on same diet as obtained in the healthy cases afforded an average respiratory quotient of only 0.679.

Now if one analyses the components of this respiratory quotient in Diabetes Mellitus one finds that far from there being evidence of a diminished oxidation/

oxidation there is in reality an increased oxidation for the intake of oxygen in the diabetic per kilogram of body weight per minute is 44% greater than in the case of the healthy individual and the output of carbon dioxide is likewise greater by 14% than in the case of the healthy individual. But the percentage increase in the case of carbon dioxide is much less than is the percentage increase in the case of the oxygen, which fact is only to be explained by the observation noted above that the combustion of carbohydrates leads to the greatest production of carbon dioxide and hence to the increase in the respiratory quotient.

A determination of the respiratory exchange is the method most free from fallacy to determine a diminution in carbon dioxide production, for such a determination not only gives the absolute value of the oxygen absorbed and of the carbon dioxide eliminated/

eliminated but also shows the relationship between the intake of oxygen and the output of carbon dioxide.

Along with this relative diminution in carbon dioxide output we find associated a diminished alkalinity of the blood but there is no accumulation of carbon dioxide in the tissues as is pointed out by Beddard, Pembrey and Spriggs,⁽⁴⁾ both facts being due to the diminished combustion of carbohydrates - lactic acid and other acids B oxybutyric acid, B amido butyric acid taking part in the reduction of the blood alkalinity. Such diminution as pointed out by the above authors, may extend from $\frac{N}{70}$ - $\frac{N}{90}$ NaOH, the average alkalinity of blood corresponding, according to Wright, to about $\frac{N}{30}$ NaOH.

The coma that is frequently the terminal phenomenon in sexes of Diabetes Mellitus is always associated with marked acidosis, which acidosis has been, from Walter's⁽⁵⁾ experiments given as the explanatory/

cases

explanatory cause of the development of coma.

Grube⁽⁶⁾ also produced coma by using B amido butyric acid to induce acidosis.

There is thus evidence that while the carbohydrates to a very large extent if not altogether escape combustion, the potential energy to produce the living force necessary for continued existence is found in the proteid and fat elements of the dietary, if waste is to be avoided.

This indeed is a wasteful process, much of the proteid escaping complete combustion and adding to the already accumulated carbohydrate in the blood, albumin being broken up in the diabetic organism into urea and glucose.

In Diabetes Mellitus therefore while there is no total diminution of oxidation in the body there is some morbid factor exercising a very definite interference with the normal consumption of the carbohydrates.

Is it conceivable that this diminished carbohydrate consumption can be due to a simple non-oxidation while the proteids and fats are still oxidised? That it may be and most probably is so can be shown by the results of several experiments.

Pfluger⁽⁷⁾ has shown that such may be the case and Straub⁽⁸⁾ and Rosenstein⁽⁹⁾ also demonstrated that animals which had been fed only upon proteids when poisoned by means of carbon monoxide gas developed glycosuria. This effect is of significance as displaying the ability of the carbon monoxide haemoglobin to partially consume the proteid but its inability to consume the glucose produced from its partial combustion.

When oxyhaemoglobin is brought in contact with carbon monoxide gas a more stable combination with this latter gas and haemoglobin is formed leading to a very marked diminution in the oxygen bearing power of/

of the haemoglobin.

Araki⁽¹⁰⁾ likewise demonstrated that animals, fed only on proteids and exposed to an atmosphere poor in oxygen (5% oxygen) developed glycosuria displaying still a power of utilising to a partial degree, the ingested proteids.

The latter author in similar experiments but upon animals fed on carbohydrates found also that glycosuria developed. The sugar eliminated was invariably glucose.

Other combinations of Haemoglobin, for example with the nitrite radicle⁽¹¹⁾ and cyanogen radicle⁽¹²⁾ such combinations again being produced at the expense of the oxyhaemoglobin and of greater stability than the latter combination, have been found to be followed by the condition of glycosuria.

Of very suggestive significance are the recorded collateral observations by several of the above quoted authors, of the diminished alkalinity of the blood/

blood and the increased elimination in the urine of salts of organic acids e.g. lactic acid.

Hammester⁽¹³⁾ also noted diminished alkalinity of blood after carbon monoxide poisoning and also after Nitrite of Amyl poisoning.

Many other substances e.g. phosphorus⁽¹⁴⁾ and arsenious acid whose pharmacological actions are in the direction of diminution of normal metabolic processes have likewise the power of producing glycosuria as well as fatty deposits and fatty degenerations.

When one recalls the ultimate relationship between carbohydrate and fat in the body this latter fact in view of the occurrence of lipaemia in Diabetes Mellitus cannot but be of some significance. This condition of lipaemia has also been described as occurring after carbon monoxide poisoning.⁽¹⁵⁾

We have also to record in support of this modified oxidation theory the observations made by Claude Bernard⁽¹⁶⁾ that a noticeable increase of sugar in/

in blood and elimination in the urine follows withdrawal of considerable quantities of blood from animals. These observations have been confirmed by von Mering and Irisawa.. Araki likewise found the same result following such losses.

Van Noorden⁽¹⁷⁾ in discussing the theories of the causation of Diabetes Mellitus concludes by taking as an insuperable objection to the non-consumption theory the fact that in cases of Diabetes Mellitus the liver, even with a full carbohydrate diet, instead of being laden with unconsumed glycogen, has a very small glycogen content.

That this low glycogen content is not incompatible with the theory of diminished consumption appears more than probable from the results of Araki's⁽¹⁸⁾ experiments who found that the glycogen content of the liver and also of the muscles was very markedly diminished in all of many animals that he exposed to carbon monoxide poisoning and likewise to atmospheres/

atmospheres poor in oxygen and also after inhaling nitrite of amyl. Urea he also noted as being increased in Carbon monoxide poisoning.

It would therefore appear that a want of available oxygen is responsible not only for the failure of glucose consumption but in some way for the failure of the maintenance of the glycogen molecule, and further a want of available oxygen can explain in addition to the non-consumption of glucose the numerous morbid conditions obtaining in the disease Diabetes Mellitus.

Blood has its oxygen in combination with haemoglobin as oxyhaemoglobin. This dissociates in the tissues liberating what oxygen is required into the plasma and so into the lymph. The oxyhaemoglobin is not all reduced in the tissues. If the union between the haemoglobin and oxygen be stronger than is normal then the tension of oxygen in/

in the blood plasma will become so low as to lead to a condition exactly analagous to the state of affairs produced in the blood by subjecting an animal to an atmosphere poor in oxygen, or, what comes to the same thing, so modifying by artificial means a large proportion of its Haemoglobin as to sensibly diminish the total oxygen bearing capacity of the blood or in other words, produce oxygen starvation of the tissues.


The blood of Diabetic patients was examined spectroscopically in the first instance to determine whether the normal spectrum was obtained. The normal spectrum was obtained in all cases and the oxyhaemoglobin reduced by the usual reagents, and methaemoglobin was formed without difficulty in the usual way. Haemin crystals could also be formed.

Further experiments were further performed, in the next instance, to determine whether the blood from these cases of Diabetes Mellitus, gave any evidence /

evidence of an abnormally diminished mobility of the oxygen in the oxyhaemoglobin molecule.

Solutions of Haemoglobin were made from blood taken from cases of Diabetes Mellitus and the mobility of the oxygen in the oxyhaemoglobin compared with the mobility of the oxygen in solutions of healthy blood. These solutions to be compared were made in tubes of equal calibre and the degree of concentration made as equal as it was possible by the naked eye assisted by the spectroscope, an intensity of colour being aimed at, where both the characteristic oxyhaemoglobin bands just merged. Equal quantities of these solutions were taken and mixed with an equal quantity of distilled water in tubes of narrow calibre. These tubes were examined in pairs by the spectroscope and the bands carefully focussed. To tubes of healthy and diabetic blood alternately were added equal amounts of oxyhaemoglobin reducing fluid. This fluid which requires to be made/

made up fresh for each series of experiments was made from the formula of Stokes.⁽¹⁹⁾ The reducing power of this solution depends on the presence in it of a very readily reducing ferrous salt which exerts a powerful and rapid^{reducing} action on oxyhaemoglobin in the cold.

The results obtained showed that in all instances
a definitely increased
there was  delay in the reduction of the
diabetic oxyhaemoglobin, a delay averaging in all
observations 74 seconds, the average reducing time
of healthy blood under the identical circumstances
being 36 seconds.

Four cases in all of Diabetes Mellitus were examined in this way and several examinations made of each case.

The cases were all of considerable severity and occurring in adults. For facilities and for permission to examine these cases I am indebted to Professor Sir Thomas R. Fraser, under whose care they were/

were.

In order to control these results experiments were performed of a similar nature, healthy blood being only used but to several tubes were added (1) glucose (2) B oxybutyric acid (3) B amido-butyric acid (4) Hydrochloric acid and (5) a mixture of glucose and B oxybutyric acid.

The amounts of these various constituents added were such that a solution was obtained where the abnormal constituent ^{(1), (2) or (3)} was present in such an amount as is found in diabetes mellitus.

In this way it was sought to determine whether the delayed reduction of the diabetic oxyhaemoglobin were due to such an abnormal constituent and in several examinations of such solutions there could not be noted any appreciable delay in reduction from the time interval occurring in the case of a simultaneous normal healthy blood determination.

The/

The amount of glucose present in the latter control tube was 0.4% that representing according to Naunyn⁽²⁰⁾ the amount present in the blood of a diabetes Mellitus of average severity. The B oxybutyric acid and B amidobutyric acid were present to the extent of 0.04%. That figure represents the amount of B oxybutyric acid estimated by Magnus Levy⁽²¹⁾ to be present in Diabetic blood.

The hydrochloric acid was added in a similar amount viz. 0.04%. (*control*).

These results then of the spectroscopic examination of Diabetic blood are of interest and of significance when one bears in mind the results of those experiments quoted to demonstrate the disturbance of metabolism of the carbohydrates when the mobility of the oxygen of the oxyhaemoglobin molecule is interfered with by such artificial means as e.g. carbon//

carbon monoxide poisoning or simply by exposing animals to atmospheres poor in oxygen.

Further investigation of this subject from the therapeutic side is demanded and results may be elicited in conjunction with these observations upon the blood which may go far in shedding light upon what must still be recognised as the darkness of the pathogenesis of Diabetes Mellitus.

CONCLUSION. Bearing in mind the significant results
upon the metabolism of carbohydrates when the oxy-
gen capacity of the Haemoglobin molecule is inter-
fered with by artificial means, the suggestive
discovery is noted that there is apparently an
increased stability of the Oxy-haemoglobin mole-
cule in the blood of individuals suffering from
Diabetes Mellitus.

B I B L I O G R A P H Y.

- (1) Minkowski, Untersuchungen ueber Diabetes Mellitus. Leipsig 1893.
- (2) Weintraud and Laves E., Zeitschrift für Phys. Chemie Band. 19.
- (3) Laves E., ibidem, Band. 19.
- (4) Beddard, Pembrey & Spriggs, Lancet May 16th 1903.
- (5) Walter, Archiv. für Experiment. Path. und Pharmakologie Band. 7.
- (6) Grube, ibidem, Band 44.
- (7) Pflüger, Archiv. für die gesammte Physiologie - Pflüger. 1903.
- (8) Straub, Archiv. für experiment. Path. und Pharmakologie Band 38.
- (9) Rosenstein, ibidem, Band. 40.
- (10) Araki, Zeitschrift für Phys. Chemie, Band. 15 & 17.
- (11) Araki, ibidem, Band. 17
- (12) Zillesen, ibidem, Band. 15
- (13) Hammester, Lehrbuch für Phys. Chemie S. 64.
- (14) Araki, Zeitschrift für Phys. Chemie, Band. 17 & 19.
- (15) Fischer, Virchow's Archiv. für Anat. und Phys. 1903.
- (16) Bernard, Leçons sur le diabete et la glycogen animale.
- (17) Von Noorden, Twentieth Century of Medicine - Diabetes Mellitus.
- (18)/

B I B L I O G R A P H Y. continued.

- (18) Araki, Zeitschrift fur Phys. Chemie, Band 19.
- (19) Gamgee Schafer's Physiology p.230. Vol.I.
- (20) Naunyn, Nothnagel's Specielle Path. und Therapie- Diabetes Mellitus.
- (21) Magnus Levy, Archiv. für Experimentelle Path. und Pharmakologie, Band.
42 & 45.

Other works consulted were

- (22) Clifford Allbutt's System of Medicine, Article on Diabetes Mellitus
by Saundby.
- (23) Pavy's Physiology of the carbohydrates.